International Journal of Trend in Scientific Research and Development (IJTSRD)
Volume 5 Issue 4, May-June 2021 Available Online: www.ijtsrd.com e-ISSN: 2456 – 6470

Experimental Study and Analysis of the Effect of Natural Microbial in Expansive Clayey Soil

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ABSTRACT

In the construction fields, the main characteristic required for the soil is their capacity to resist the super imposed loads safely by a control in stresses and deformations. Among the different types of soils, the construction activities on expansive soils requires adequate care since it was a fine grained soil that shows large volume change when exposed to varying water content.

According to various studies, sub grade stabilization in roads can provide significant improvement in engineering properties. Microbial stabilisation is a new and alternative treatment method in geotechnical field by the use of bacteria in soil improvement, which are already naturally present in soils to develop more by providing the nutrients as an energy source. So far the researcher's has been carried out with Microbial induced calcite precipitation (MICP) bacteria for the production of calcium precipitates by biological method and have practiced only on the sandy soils. These researches were done on the Experimental Study And Analysis Of The Effect OF Natural Microbial In Expansive Clayey Soil forming a cementious soil matrix.

Totally 405 samples were prepared under varying bacterial concentrations of 106, 107 and 108 cfu/ml, incubation periods of 1, 3, and 4 days with increasing curing periods of 1,7,14, 28 and 60 days. For each set of test 3 specimens were tested to evaluate the accurate value.

For the volume changing nature of clay soil were analysed by conducting the Free Swell Index (FSI) tests. From the FSI test, the swell got decreased from 85% to 30% for Acetobacter treated soil. Similarly the free swell values are also decreased to 20% for soil treated with Acetobacter and 35% for the soil treated with E.coli at 60 day age curing, 108 cfu/ml concentration and 4 day incubation period, as compared with untreated soil.

The peak stresses for the 60 days curing period for Acetobacter, Bacillus and E.coli bacteria are

410 kPa, 521 kPa and 341 kPa respectively. As the curing period increases the UCS value of Bacillus improved more than Acetobacter and E.coli at 60 day age and with 108 cfu/ml concentration. The UCS strength of Bacillus bacteria treated soil was 4.5 times greater than the untreated soil strength when compared with Acetobacter and E.coli.

For the CBR tests on treated soils, Acetobacter, Bacillus and E.coli were mixed with soil in varying concentrations (ie, 106, 107 and 108cfu/ml) with incubation periods 1, 3, and 4 days and tested after curing the samples for 60 days to know the effect of long term treatment. California Bearing Ratio of soil increases to 6.7%, 8.2% and 5.9% respectively after 60 days curing from a value 2.3% with Acetobacter, Bacillus and E.coli and the concentrations of 108cfu/ml and 4 day incubation period

KEYWORDS: *Expansive Clayey Soil, Microbial stabilization, water content, engineering properties, bacterial concentrations, MICP*

How to cite this paper: Rishav Sharma | Prof. Nitesh Kushwaha "Experimental Study and Analysis of the Effect of Natural Microbial in Expansive Clayey Soil" Published in International Journal of Trend in Scientific Research and Development (ijtsrd), ISSN: 2456-6470, Volume-5 | Issue-4, June 2021, pp.451-464, URL: www.ijtsrd.com/papers/ijtsrd42332.pdf

IJTSRD42332

1. INTRODUCTION

Evolving civilization plays a key parameter in improving the weak grounds and making them useful. Proposed and existing structures completely relay upon the type and strength of foundations which are an inevitable constraints. The structure remains healthy as the foundation strength is improved and becomes appropriate. Therefore, the study and analysis of soil with its index and engineering properties are essential to conclude the foundation details and other parameters. Natural soil has both a complex and variable material and it has to be considered as the basic engineering material to meet alltypes of construction activities.

The classic problem in Civil Engineering is the low bearing capacity of naturally available soil, when it is necessary to

prepare a subgrade to construct lightweight structures, pavements, railroads, runway etc. The subgrade is mainly required to provide a geometric regularity and transmit the load properly to its underlying strata. Among the available soil for the construction, an outstanding problem has been involved in the strength of fine grained soils and the enhancement on instability of its volume with the variation of moisture content. The major problems such as the low bearing capacity and high compressibility, becomes a challenging issues by the Civil Engineers. To suggest a suitable geotechnical design of structure, it requires a detailed soil feasibility studies to fix the characteristics of the subsoil.

The undesirable soil properties results in abandoning of site will demands for naturally available resources and availability of areas. For the construction activities on weak soils are uncertain, mainly leading to the availability of increasing scarcity of land, but the rapid increase in industrialization, demands more for available areas for various activities even if it is unsuited, wholly or partially to meet the constructions. Hence, this demands in improving the properties of soil by altering or using ground improvement methods.

The present scenario and circumstances need to improve the available weak soil deposits by modifying their characteristic properties. Even, the majority of poor soils like organic peat and soft clays could be treated by adopting various ground improvement methods. The stabilization of soil with various admixtures are the most economical and less tedious method, which are adoptable. The soil stabilization process increases the bearing parameters and shear strength characteristics of soil thus reducing settlement failures and offering economical foundation design. It increases the resistance offered by the soil to softening and volumetric changes in presence of water by thorough bonding between the soil grains and also providing water shielding in the combined state with the addition of a modifier to the soil for specific engineering projects. The main concern of geotechnical and civil engineers to provide solutions having an economical design is to stabilize and sustain the soil. Thus the soil alteration mainly enables the suitability of soil for construction beyond their original capacities. The primitive and the simplest stabilization processes are compaction (removal of air voids) and dewatering (draining out excess water) makes the soil stronger.

2. PROBLEMS IDENTIFICATION AND OBJECTIVES

2.1. PROBLEMS IDENTIFICATION

Based on the literature studies, the need for soil properties improvement within the subsurface environmental factors helps in stabilization of problematic soil with an eco-friendlier and sustainable ground improvement method. In the microbial stabilization methods, so far it has reported better suitability in cohessionless soil and concrete. Hence, in this research, stabilization of expansive soil can be done by using bacteria which is non-toxic and eco-friendly. The availability of different chemical based admixtures, this work gives special emphasis to microbial effect with clay soil particles to improve its strength. Thus the present study is to carry out a feasibility study pertaining to the utilization of Microbial Induced Calcite Precipitation (MICP) to embrace the further characteristics of expansive soil which is problematic in nature, having high compressibility and swelling properties and inadequate bearing capacity.

2.2. OBJECTIVES OF STUDY

The objectives of the present study are formulated as below:

- To study the consequence of Microbially Induced Calcite Precipitation (MICP) on strengthcharacteristic of soils.
- To find out optimum bacterial cell concentration and cementation reagent (MICP) for the strength improvement.
- To study the improvement of California Bearing Ratio (CBR) and Free Swell Index of MICPtreated soil.
- To study the influence of curing period on treatment of soil with MICP.

3. METHODOLOGY

3.1. GENERAL

The effect of industrialization obsoletes the available suitable land and urges the necessity of stabilizing the soil to make them expedient. The extinction of good soil promotes treatment of weak and poor soil suitable for various constructional activities. Usage of various admixtures in soil helps in attaining an improvement in properties. The inclusion of bacteria helps in attaining better improvement in strength characteristics. The soil sample to be treated is collected from particular depth by abiding the procedure and is tested for virgin soil properties. The bacteria which are involved in the treatment are cultured through a nourished medium. The basic properties and characteristic feature of bacteria can be explored. The study focuses on progress in the compressive quality of fine-grained soil with Microbiologically Induced Calcite Precipitation (MICP). Study on various types of bacteria and micro-structural analysis by using SEM for the arrangement of particles and cementitious formation after treatment are briefly explained with the morphological studies. The X-Ray Diffraction techniques confirmed the elements behind the cementitious formations. Basic tests are conducted on the soil sample collected to determine the optimum bacterial cell concentration and cementation reagent (MICP) for highest strength increment. The effect of MICP on performance of UCC, CBR; Free Swell Index of the treated soil and the effect of curing period on treatment of soil with MICP is also studied.

3.1.1. MATERIALS USED

The soil sample was collected from Bhopal, M.P., India from a depth of about 0.6 m below the ground level. The details of the sample collection locations are given in the Figure 4.1. In this area, the soil is exhibited with high swell and shrinkage characteristics and it was observed that many of the buildings were subjected to differential settlement leading to crack formation. The soil was collected and dried for few days to attain dry state and was pulverized to attain powder form. Three types of bacteria which are cultured and used in this study at different dilution factors at various growth periods. The bacteria were identified for this study from soil samples collected from Taramani. Identification of calcite precipitation and type of bacteria from soil sample site, the type cultures were used for this study. For biochemical characterization of bacteria, chemicals were purchased from Sisco Research laboratory, India. All the bacteria were purchased form microbial type culture collection centre, Chandigarh, India.

4. METHODOLOGY

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5. RESULTS AND DISCUSSION

5.1. GENERAL

In geotechnical fields, the ground improvement techniques can improve the performance of problematic soils and strength characteristics. The method of soil stabilization is one of the ground improvements which can be performed using the MICP bacteria. In this study, *Acetobacter, Bacillus* and *E.coli* bacteria of different concentrations were used for the stabilization of the problematic soil. Effectiveness of the usage of the MICP bacteria on soil is evaluated by conducting the strength characteristics, free swell index and micro structural analysis and elemental composition were carried out using SEM and XRD experimental results respectively.

5.2. UCC TEST RESULTS ON UNTREATED AND TREATED SOILS

The Un Confined Compression (UCC) tests on soil were carried out as per the Bureau of Indian Standard (BIS) code procedure. The samples were prepared by compacting the sample in split mould. While compaction, greezing was done for easy removal of sample [132]. In case of virgin soil, after removal of the soil sample from the mould the test was conducted. In this study, different concentrations of *Acetobacter, Bacillus* and *E.coli* bacteria were used to treat the virgin soil sample. The soil samples were placed in polythene covers and covering with wetted gunny bags for curing the samples [6]. Curing was done and tested for 1, 3, 7, 14, 28 and 60 days until failure. As per IS: 2720 (Part 10) [122], an axial strain rate of 1.25mm/min were used to test the all the untreated and treated soil specimens. For all the tests, three samples for all the combinations of soil and bacteria were prepared, with the aim of providing sufficient results for accurate interpolation to analyse the results of UCC values of untreated and treated soil samples. For the UCC tests for treated soil with *Acetobacter, Bacillus* and *E.coli* a total of 405 samples were prepared under varying concentrations of 10^6, 10^7 and 10^8 cfu/ml, incubation periods of 1, 3, and 4 days with increasing curing periods of 1, 7, 14, 28 and 60 days. For each test set of three specimens were tested to evaluate the accurate value.

5.2.1. Stress Strain Characteristics of Treated Soil

To study the strength characteristics with bacteria on soil improvement, the UCC tests were conducted with three different bacteria with various concentrations at different incubation and curing periods. Samples are treated with varying concentrations of *Acetobacter, Bacillus* and *E.coli* bacteria.

The treated samples were placed in air tight bags and allowed for curing for the periods of 1, 7, 14, 28and 60 days and after, the sample were tested to obtain the Unconfined Compressive Strength (UCS) values. The peak stress values obtained for the soil treated with *Acetobacter* at different curing ages are presented in Table 5.1.

Table 5.1 Unconfined compressive strength value of soil treated with *Acetobacter*

Age of bacteria		Unconfined Compressive Strength of Soil (kPa)								
		Day (1D)			Days (3D)			Days (4D)		
fu/ml		10^6	0^7	0^8	0^6	0^7	0^8	0^6	10^7	10^8
U ring P Days)	1	175	84	13	86	04	20	04	215	230
	7	179	86	20	18	21	40	32	247	252
	14	221	28	45	33	40	54	50	259	289
	28	248	55	59	70	83	15	85	294	335
	60	286	98	49	93	30	92	32	339	410

It was seen that the compressive strengths test conducted on treated soils are greatly developed compared with the strength of untreated soil sample. The modification of the unconfined compressive strength with respect to curing time for soil sample and it influences with bacteria stabilizers on the strength gain of the treated soils are noticed. The strength development is mainly due to the reactions of bacteria with higher cfu/ml and increase in curing periods The stress versus strain graphs are plotted for all the soil samples treated with different concentrations of *Acetobacter, Bacillus* and *E.coli* bacteria. Figures

5.3. 5.1.-5.3 show the stress-strain behavior of treated soils with concentration of 108 cfu/ml Acetobacter.

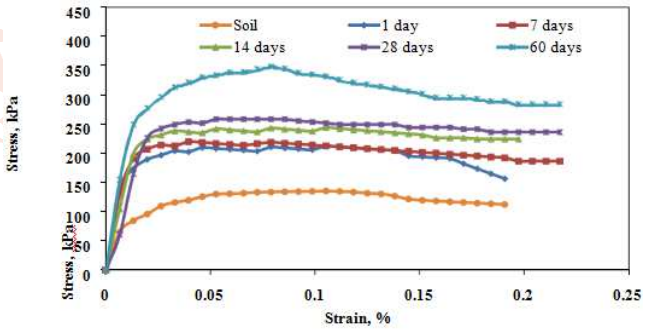

Figure 5.1 Stress strain behaviour of soil treated with 1 day age of *Acetobacter* with10^8 cfu/ml for various curing period.

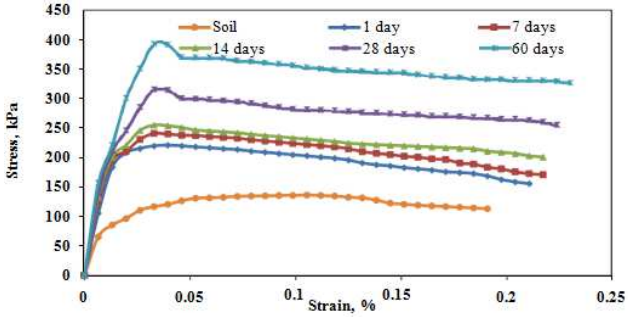

Figure 5.2 Stress strain behaviour of soil treated with 3 days age of *Acetobacter* with 10^8 cfu/ml for various curing period.

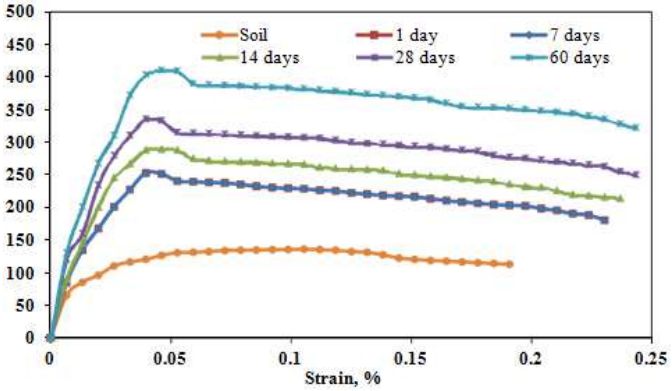

Figure 5.3 Stress strain behaviour of soil treated with 4 day age of *Acetobacter* with 10^8 cfu/ml for various curing period

Similarly *Bacillus* and *E.coli* bacteria were isolated from cemented soil and treated with soil which is of varying incubation period with different concentrations. Figure 5.4 and 4.5 shows the stress strain behavior of treated soils with different concentrations of *Bacillus* and *E.coli* bacteria which are of age 4 day at 10^8 cfu/ml concentration. The values of peak stresses and corresponding strain values of soil treated with *Bacillus* and *E.coli* bacteria are presented in Table 5.2 and 4.3.

Table 5.2 Unconfined compressive strength value of soil treated with *Bacillus*

Age of bacteria		Unconfined Compressive Strength of Soil (kPa)								
		Day (1D)			Days (3D)			Days (4D)		
fu/ml		10^6	0^7	0^8	0^6	0^7	0^8	0^6	10^7	10^8
uring P Days)	1	84	84	13	86	04	20	04	215	230
	7	86	86	20	18	21	40	32	247	252
	14	28	28	45	33	40	54	50	259	289
	28	55	55	59	70	83	15	85	294	335
	60	98	98	49	93	30	92	32	339	410

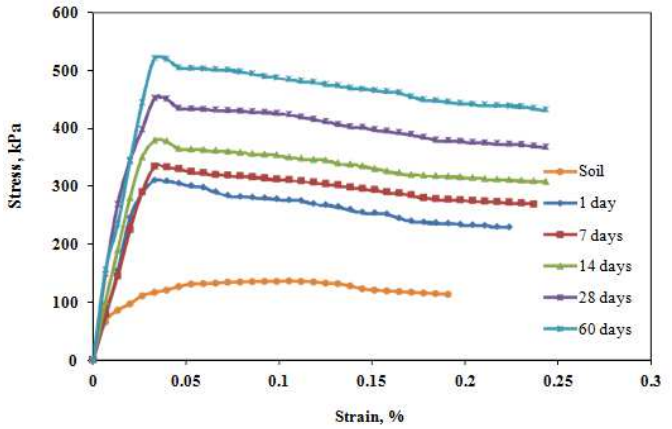

Figure 5.4 Stress strain behaviour of soil treated with 4 day age of *Bacillus* with 10^8 cfu/ml for variouscuring period

Table 5.3 Unconfined compressive strength value of soil treated with *E. coli*

Age of bacter (Days)		Unconfined Compressive Strength of Soil (kPa)								
		Day (1D)			Days (3D)			Days (4D)		
fu/ml		10^6		0^8	0^6	10^7	0^8	10^6		10^8
uring Pe Days)		154		94	66	179	94	190		210
		171		11	84	200	20	201		232
	4	193		34	10	229	47	223		261
	8	234		78	38	274	93	255		298
	0	259		94	65	300	34	298		341

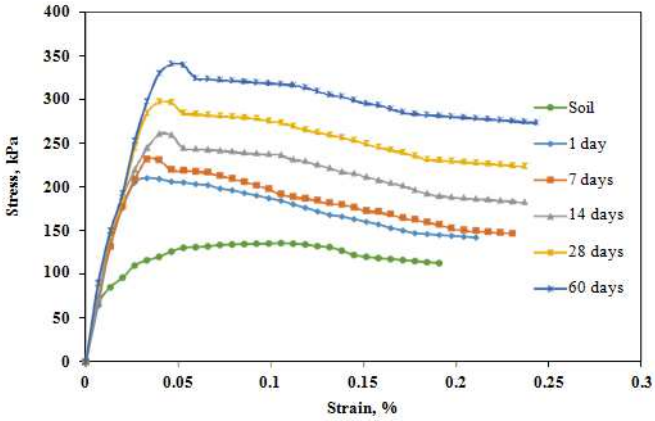

Figure 5.5 Stress strain behaviour of soil treated with 4 day age of *E. coli* with 10^8 cfu/ml for variouscuring period

From the stress strain graphs, a same trend as compared with the stress strain variation when soil treated with *Acetobacter* was observed. That is, the stress values increased rapidly with lesser strain and further reach the sustaining value. The peak stresses for the 60 days curing period for *Acetobacter*, *Bacillus* and *E.coli* are 410 kPa, 521 kPa and 341 kPa, respectively. In all the cases of bacterial treatment with the soil the peak stress and the stable residual phases are seen distinctly. The peak stress and residual stresses are increased with increase in bacterial concentration and incubation periods. The failure mode of untreated soil sample and soil treated with bacteria are shown in Figure 5.6.

5.2.2. Effect of Bacterial Concentration on Treated Soil

The influence due to the bacterial concentration on treated soils shows the increase in stress and the MICP bacteria age. Figures 5.7 - 5.9 clearly indicates that the stress is generally increase rapidly and reached the peakand decreased at 60 days of curing period condition, exhibits the failure nature of the specimen is brittle.

Figure 5.6 Failure modes unconfined compressive strength specimens of untreated and bacteria treated soil

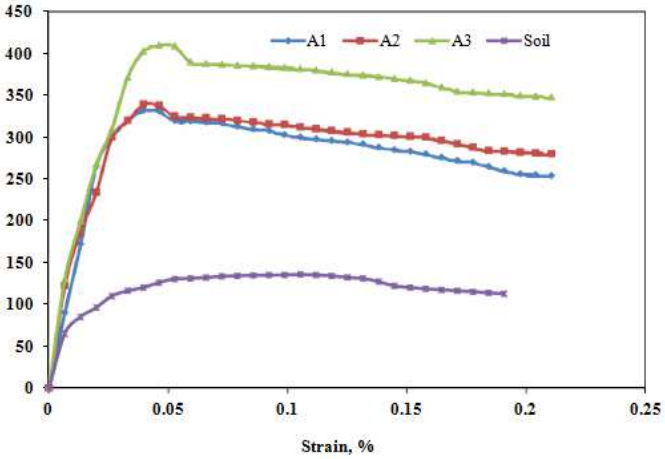

Figure 5.7 Stress strain behaviour of soil treated with 4 day age of *Acetobacter* for various concentrationat 60 days curing period (A1 - 10^6, A2 – 10^7, A3 -10^8 cfu/ml)

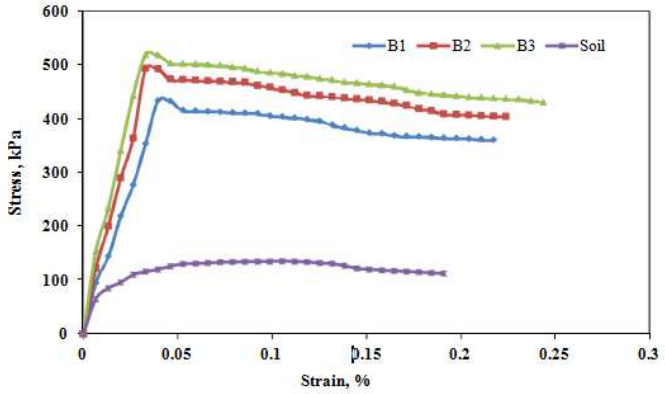

Figure 5.8 Stress strain behaviour of soil treated with 4 day age of *Bacillus* for various concentrations at60 days curing period (B1 - 10^6, B2 – 10^7, B3 -10^8 cfu/ml).

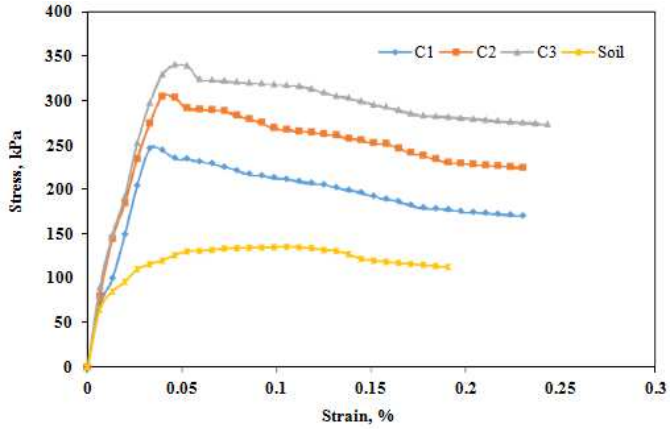

Figure 5.9 Stress strain behaviour of soil treated with 4 day age of *E.coli* for variousconcentrations at 60 days curing period (C1 - 10^6, C2 – 10^7, C3 -10^8 cfu/ml).

For the initial curing periods, as the failure of the specimen is of ductile nature, the increment in stress observed is linear. The variation of unconfined compressive strength value under incubation periods 1, 3 and 4 days and concentration of 10^6, 10^7 and 10^8 cfu/ml when treated with soil are presented and discussed. The typical variation peak unconfined compressive strength values for the tests conducted on treated soil with *Acetobacter*, *Bacillus* and *E.coli* bacteria at 60 days curing period are shown Figure 5.10 - 4.12.

From the Fig 5.10 - 5.12, it can be noted that, the increase in concentration of the bacteria increases the unconfined compressive strength value. This is because, as the concentration of the bacteria increases, the growth in the number of cells of bacteria increases which increases the reaction rate with the soil and there by increases the cementation production in the soil.

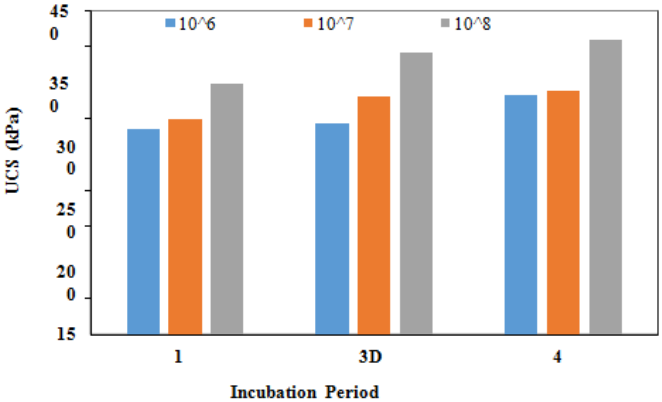

Figure 5.10 Influence of bacterial concentration on unconfined compressive strength value of soil treated with *Acetobacter* for various incubation periods.

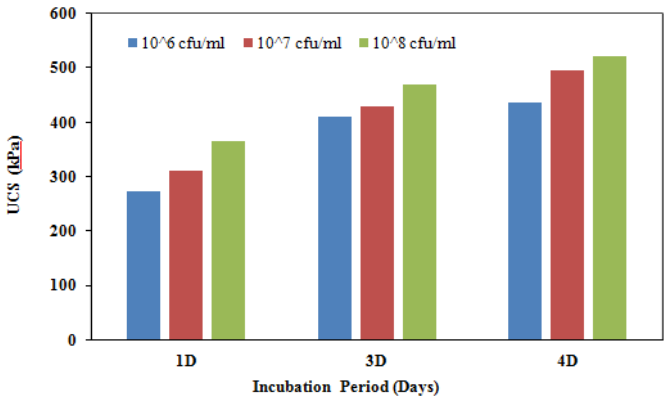

Figure 5.11 Influence of bacterial concentration on unconfined compressive strength value of soil treated with *Bacillus* for various incubation periods.

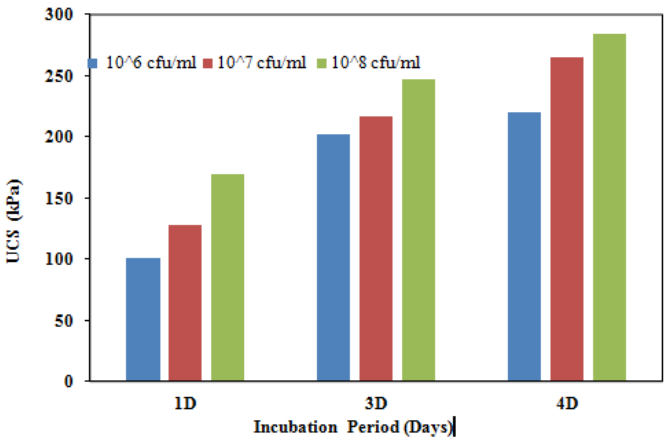

Figure 5.12 Influence of bacterial concentration on unconfined compressive strength value of soil treated with *E. coli* for various incubation periods.

5.2.3. Effect of Incubation Period on Treated Soil

The effect of age of bacteria on the treated soil is determined from unconfined compressive strength results. It can be seen that the unconfined compressive strength of clay soil treated with different concentrations of *Acetobacter*, *Bacillus* and *E.coli* bacteria was improved. For comparison, the peak unconfined compressive strength value of treated soil using the bacteria at different incubation periods and varying concentrations are shown in Figures 5.13 – 5.15.

From the experimental results it is observed that, as the age of bacteria increases, the treated soil strength also increases. This is because, as the bacteria is incubated for 24h, the growth in the cells is increased, known as logphase of growth. The increase of the growth of bacterial cells is upto 4 days known as stationary phase. As the growth of cells is more at 4 days incubation, the calcium precipitation is more when it is reacted with soil. Therefore the strength obtained at age of 4^{th} day of bacteria is more than that at the age of 1^{st} day bacteria.

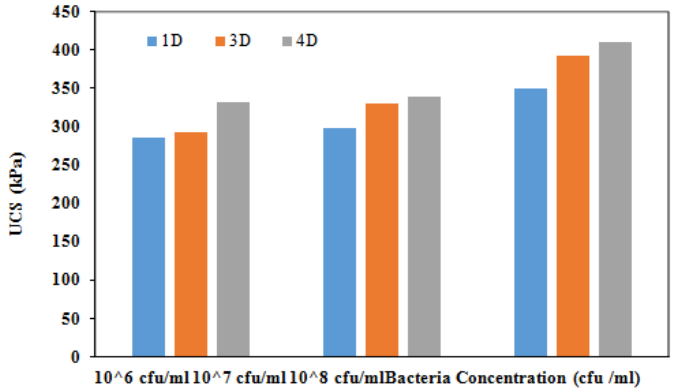

Figure 5.13 Influence of incubation period on unconfined compressive strength value of soiltreated with *Acetobacter* for various concentrations.

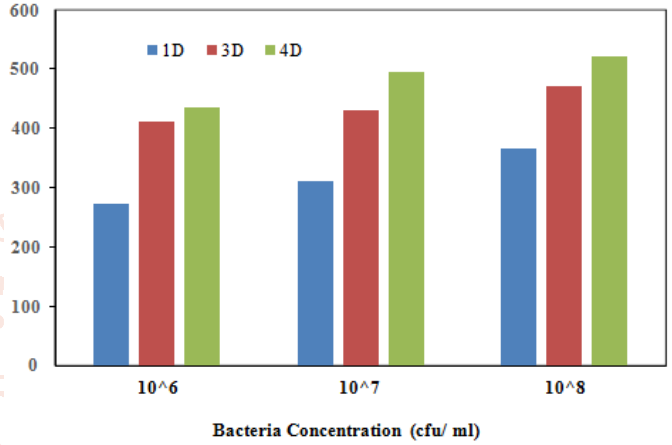

Figure 5.14 Influence of incubation period on unconfined compressive strength value of soil treated with *Bacillus* for various dilution factors.

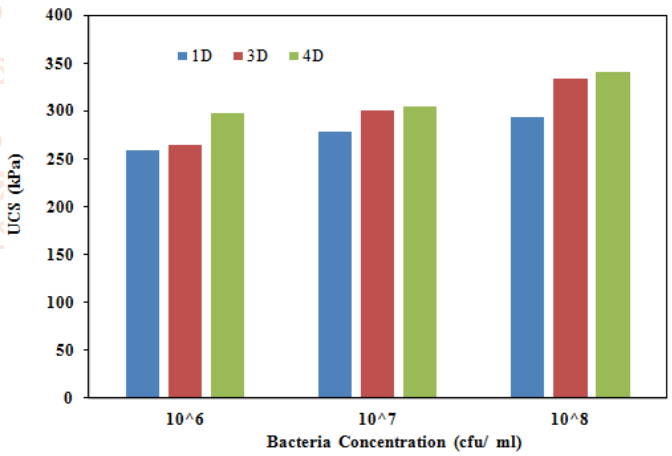

Figure 5.15 Influence of incubation period on unconfined compressive strength value of soil treated with *E. coli* for various dilution factors.

5.2.4. Effect of Curing Period on Treated Soil

The outcome of untreated soil obtained from unconfined compressive strength test showed that, there was improvement in the unconfined compressive strength of soil treated with different concentrations of *Acetobacter*, *Bacillus* and *E.coli*. The strength developed from unconfined compressive strength for different curing period are shown in Figures 5.16 - 5.18. There was rise on strength with increase in curing period. The increase is more with respect to curing period and in the initial stage, it is more and later stages, it is less. It is more in high concentration compare to the lesser concentration. The percentage improvement in unconfined compressive strength values were shown in Table

5.4 - 4.6 for the soil treated with *Acetobacter*, *Bacillus* and *E. coli*.

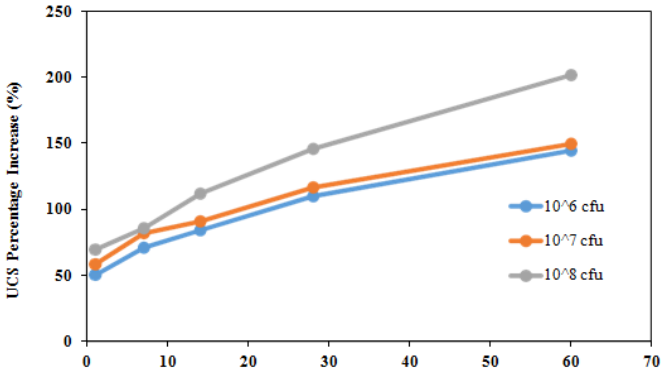

Figure 5.16 Effect of curing period on strength development of soil treated with

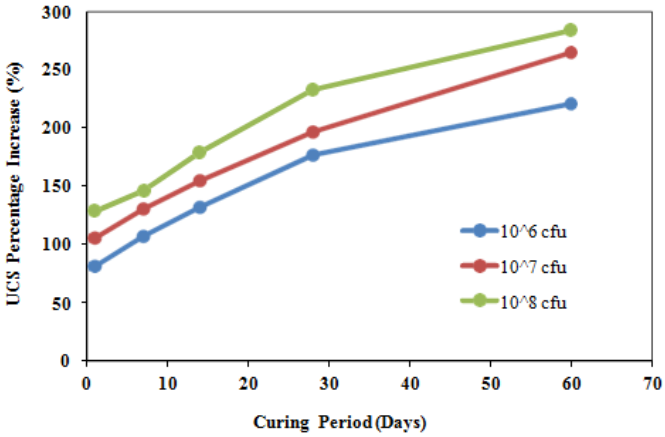

Figure 5.17 Effect of curing period on strength development of soil treated with *Bacillus* for various dilution factors

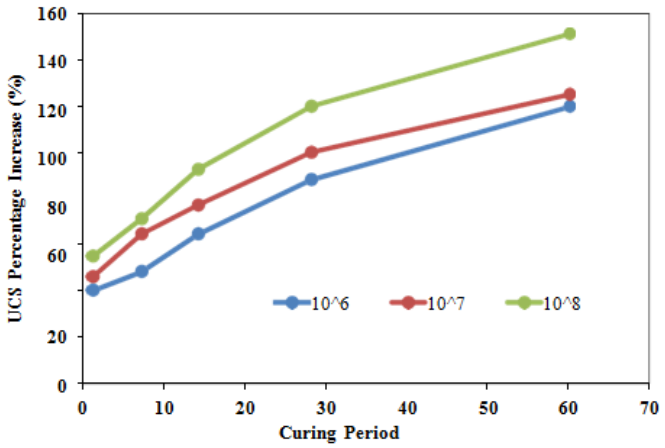

Figure 5.18 Effect of curing period on strength development of soil treated with *E. coli* for various dilution factors

Table 5.4 Percentage improvement of unconfined compressive strength value of soil treated with *Acetobacter*

Age of bacteriaDays)	improvement of UCS Value (%)							
	Day (1D)		Days (3D)			Days (4D)		
fu/ml	0^6	0^7	10^6	10^7	10^8	0^7	10^8	
uring Per Days)			37.1	50.3	62.1	8.4	69.5	
			60.6	62.9	76.8	1	2	85.7
		68	71.7	76.8	87.2	0.9	112	
			98.9	108	132	16.7	146.9	
			115.	143.	188.	49.8	202	

Table 5.5 Percentage improvement of unconfined compressive strength value of soil treated with *Bacillus*

Age of bacter (Days)		Improvement of Unconfined Compressive Strength Value (%)								
		Day (1D)			Days (3D)			Days (4D)		
fu/ml		10^6	10^7	10^8	0^6	10^7	10^8	10^6	10^7	10^8
uring Pe Days)	1	58.4	68.8	87.9		96.8	110.	80.5	104.	128.
	7	71.0	89.4	102.		119.	135.	106.	129.	146.
	14	81.3	98.2	109.		127.	143.	131.	154.	179.
	28	96.8	107.	128.		156.	183.	176.	196.	233.
	60	100.	128.	169.		216.	246.	220.	264.	283.

Table 5.6 Percentage improvement of unconfined compressive strength value of soil treated with *E.coli*

Age of bacteria		Improvement of Unconfined Compressive Strength Value (%)							
		Day (1D)			Days (3D)		Days (4D)		
fu/ml		0^6	0^7	10^8	10^7	10^8	10^6	0^7	10^8
uring Per Days)	1	3.5		43.0	31.9	43.0	40.0	5.9	54.8
	7	6.0		55.5	47.4	62.1	48.1	4.3	71.0
	14	2.2		72.4	68.8	82.0	64.3	6.9	92.3
	28	2.4		104.	101.	115.	87.9	9.7	119.
	60	0.9		116.	121.	146.	119.	24.8	151.

The experimental results show that, the unconfined compressive strength value of treated soil sample is increased gradually up to the curing period of 28 days and after that there was a rapid increase in the long term period of up to 60 days. As the curing period increases, calcium precipitation takes place, with increase in unconfined compressive strength. This indicates the increased presence and decomposition of calcium silicate and calcium aluminate hydrate in the treated soil samples [133, 134]. As the age of bacteria increases the unconfined compressive strength values increases and the increase were based on curing period and is independent of bacteria of different concentrations.

5.2.5. Effect of Various Bacteria for Various Incubation Period

The effect of different bacteria on treated soil is determined by comparing the unconfined compressive strength results. From the results it is observed that, the unconfined compressive strengthvalue is increased as the age of bacteria increases. To know the effect of type of bacteria in soil treatment, the study were made with the comparison of *Acetobacter*, *Bacillus* and *E.coli* bacteria with the specified agingand dilution factor or concentration shown in Figure 5.19.

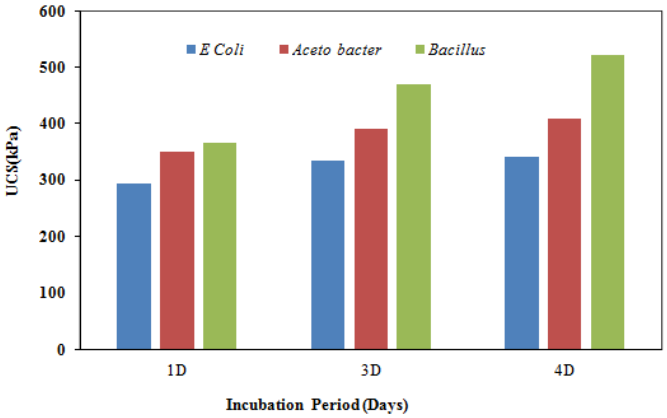

Figure 5.19 Effect of bacteria on unconfined compressive strength value of soil treated forvarious incubation period at 60 days curing period with 10^8 cfu/ml concentration

From the Figure 5.19, it is observed that, as the curing period increases the unconfined compressive strength value increases and it is observed that *Bacillus* bacteria improve the strength of the soil comparatively more than *Acetobacter* and *E.coli* bacteria at 60 day age and with 10^8 cfu/ml concentration. In case of *Bacillus* treated soil the unconfined compressive strength was 4.5 times greater than the untreated soil strength when compared with *Acetobacter* and *E.coli* bacteria.

The combination of bacterial concentration and incubation periods in soil sample provides us with the optimum pore size for the movement of bacteria through the soil composite. In the soil the higher dosage of bacterial concentration relatively higher increment in unconfined compressive strength values, this can be attributed to finer particle size of soil which provided a dense arrangement of particles and offered more particle to particle contact for the cementitious formation. It further offered higher bond formation in soil then lead to increase in cohesion of soil which is one of the parameters for the soil shear strength and hence increase in strength [78, 87].

5.3. CALIFORNIA BEARING RATIO TEST RESULTS FOR UNTREATED AND TREATEDSOILS

The CBR tests on treated soils are carried out as per BIS procedure for the soil samples mixed with bacteria *Acetobacter*, *Bacillus* and *E.coli* of varying concentration (i.e, 10^6, 10^7 and 10^8 cfu/ml) with incubation periods 1, 3, and 4 days. In the moulds, CBR samples were initially compacted to the corresponding maximum dry density obtained from the proctor compaction test results. Then, it was placed over wetted rice husk base and covered with wet gunny bags to maintain room temperature in order to avoid moisture loss from the samples prepared. After a soaking period of 4 days, the samples were tested to determine the CBR values of treated soils. In order to give adequate time to chemicals for reaction and allow development of calcite precipitates, treatment duration was provided. The samples were maintained at the temperature between 27 - 30°C. CBR specimens prepared in the mould under the curing is shown in Figure 5.20.

Figure 5.20 Curing of CBR specimen prepared with soil and bacteria

For the CBR tests for treated soil with *Acetobacter*, *Bacillus* and *E. coli* bacteria, totally 27 samples were prepared under varying concentrations, incubation periods of 1, 3, and 4 days and tested after 4 days of soaking period.

5.3.1. Load penetration characteristics of treated soil

The CBR samples prepared with the varying concentrations of bacteria on soil are allowed for the curing as per the procedure of curing the sample discussed earlier. After the curing periods, as per the Bureau of Indian Standards the CBR tests were conducted on the soil samples. The load-penetration curve for all the soil treated with various bacteria for the different concentrations were plotted and shown in Figure 5.21 to 4.23. The values of CBR of treated soil at corresponding incubation period and concentration of bacteria of *Acetobacter*, *Bacillus* and *E.coli* at the 4 days soaking period are increased.

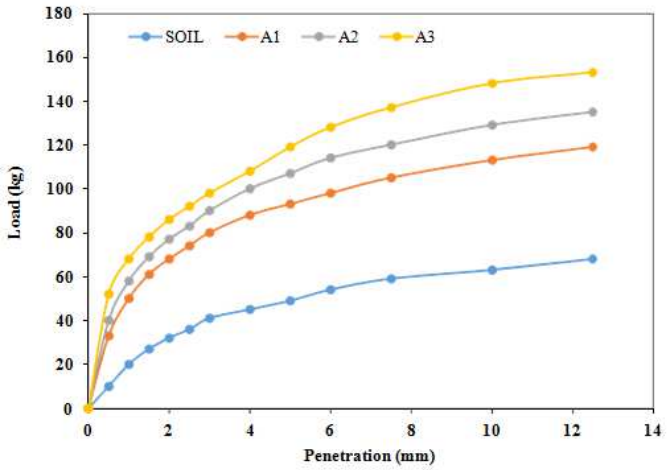

Figure 5.21 Load – Penetration curve for the soil treated with 4 day age of *Acetobacter* for various dilution factors at 60 days curing (A1 - 10^6, A2 – 10^7, A3 -10^8 cfu/ml)

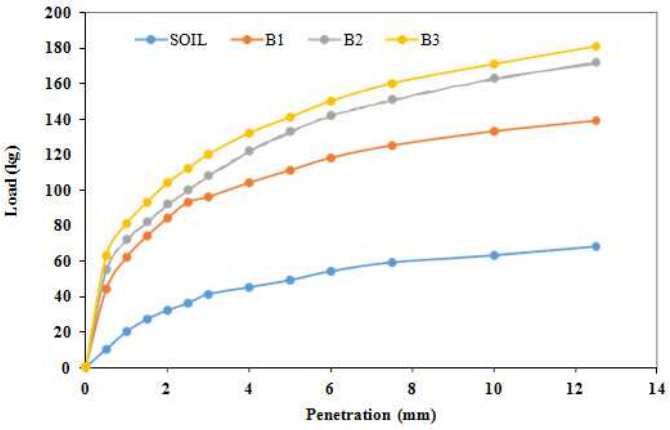

Figure 5.22 Load – Penetration curve for the soil treated with 4 day age of *Bacillus* for various dilution factors (B1 - 10^6, B2 – 10^7, B3 -10^8 cfu/ml)

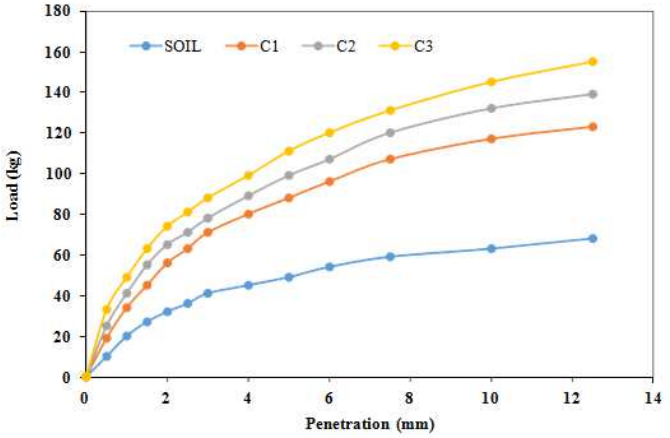

Figure 5.23 Load – Penetration curve for the soil treated with 4 day age of *E.coli* for various dilution factors (C1 - 10^6, C2 – 10^7, C3 -10^8 cfu/ml)

5.3.2. Effect of Bacterial Concentration on CBR Value of Treated Soil

From the CBR test conducted on soil with *Acetobacter*, *Bacillus* and *E. coli* bacteria with the variationin cfu/ml and incubation period is summarized and shown in Table 5.7.

Table 5.7 CBR value of soil treated with *Acetobacter*, *Bacillus* and *E. coli* bacteria

me of the bacteria		alifornia Bearing Ratio of Soil (%)								
		cetobacter (A)			*acillus* (B)			*. coli* (C)		
fu/ml		10^6	10^7	10^8	10^6	10^7	10^8	10^6	10^7	10^8
ge of acteria (Da	D	4.5	5.2	5.7	5.7	6.3	7	3.8	4.4	4.9
	D	4.9	5.5	6.2	6.1	6.9	7.5	4.3	4.8	5.5
	D	5.4	6	6.7	6.8	7.3	8.2	4.6	5.2	5.9

From the experimental values it was observed that CBR of soil increases to 6.7%, 8.2 % and 5.9%,
respectively after 4 days curing from a value 2.6% with the bacteria of *Acetobacter*, *Bacillus* and *E.coli* with 10^8cfu/ml concentration and 4 day incubation period. Increase in bacterial concentration increases the CBR values for different incubation periods. The increment in CBR may be because of the gradual formation of hydration compounds in the soil due to the reaction within the bacteria and the elements present in the clayey soil. Soil with the addition of MICP bacteria, leads to increase in more well-graded size distributions, which allows the particles to pack more closely, resulting in the increase in the density of soil [13, 15]. The CBR values increased majorly with increase with *Acetobacter*, *Bacillus* and *E.coli* bacterial concentration for the soil samples as shown in Figure 5.24to 4.26.

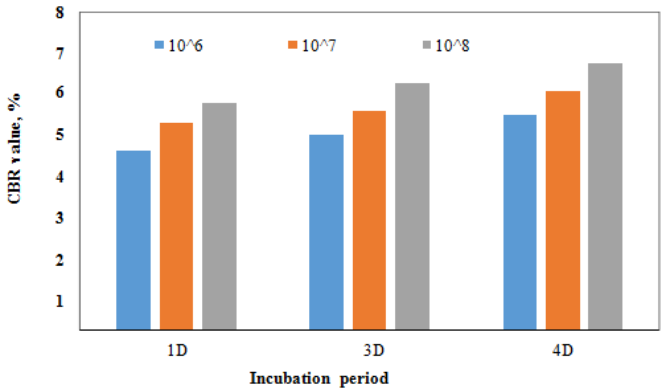

Figure 5.24 Influence of bacterial concentration on CBR value of soil treated with *Acetobacter* forvarious incubation periods

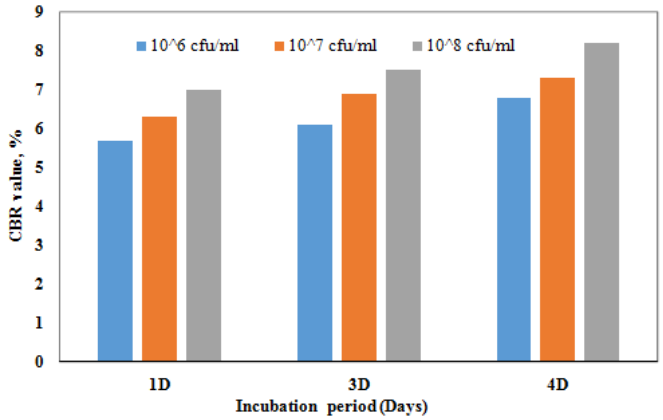

Figure 5.25 Influence of bacterial concentration on CBR value of soil treated with Bacillus for various incubation period

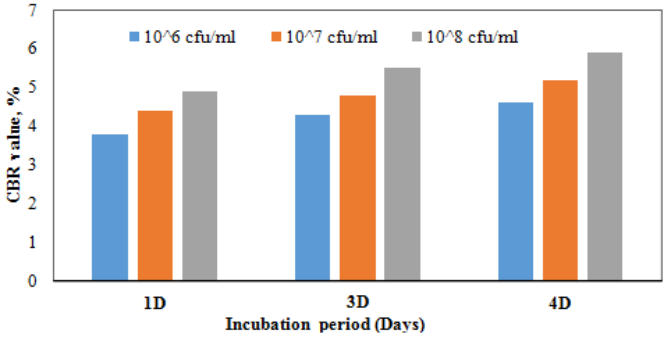

Figure 5.26 Influence of bacterial concentration on CBR value of soil treated with E. coli forvarious incubation periods

5.3.3. Effect of Incubation Period on CBR Value of Treated Soil

The effect of incubation period on treated soil was also determined by CBR test and it can be seen that the CBRvalue of soils treated with bacteria of *Acetobacter*, *Bacillus* and *E.coli* were improved. The strength developed for treated samples for various incubation periods are shown in the Figure 5.27 to 4.29. The variation in strengthindicates that, the gain in CBR increases with the increase in incubation period and bacterial concentration for the soil samples.

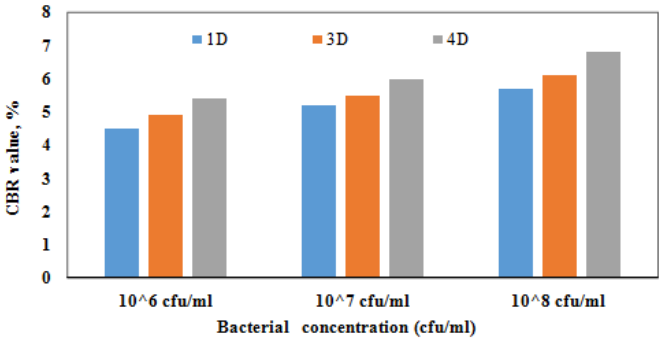

Figure 5.27 Influence of incubation period on CBR value of soil treated withAcetobacter for various concentrations.

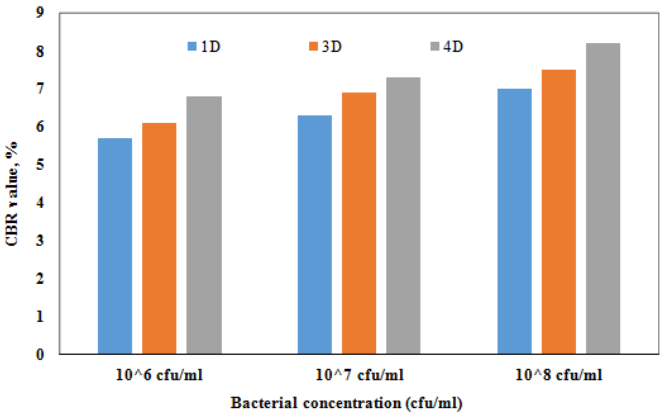

Figure 5.28 Influence of incubation period on CBR value of soil treated with *Bacillus* for various concentrations.

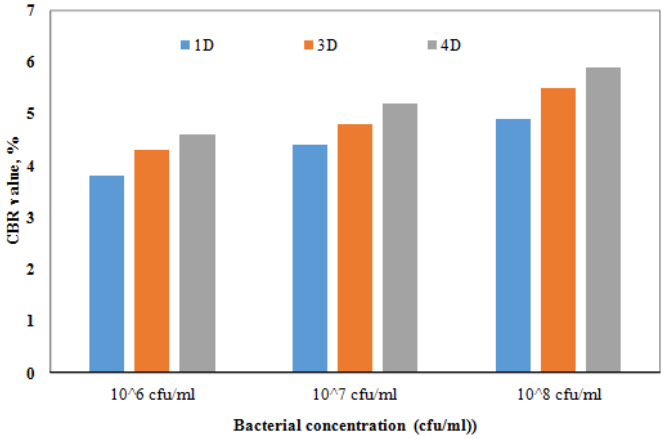

Figure 5.29 Influence of incubation period on CBR value of soil treated withE.coli for various concentrations.

It can be inferred from Figure 5.27 to 4.29 that, the bacterial addition in soil improved the California Bearing Ratio of soil sample noticeably, with a percentage increase of 157.6%, 215.38% and 126.92%,respectively for *Acetobacter*, *Bacillus* and *E.coli* bacteria with concentration of 10^8 cfu/ml and 4 days incubation period gave the optimum results.

5.3.4. Effect of Various Bacteria on CBR Value for Various Incubation Period

From the CBR test results, the CBR value for the bacteria at 60 days curing period were calculated forthe soil sample. The maximum increase in CBR value for the soil treated with *Acetobacter*, *Bacillus* and *E.coli* bacteria corresponding to 10^8cfu/ml concentration at 4 days incubation period were obtained after 4 days soaking period. The effect of type of bacteria on soil treatment, the study was made with the comparison to *Acetobacter*, *Bacillus* and *E.coli* with the specified aging and dilution factor shown in Figure 5.30.

The increase in increment of CBR value of treated soil confirms that the development of strength characteristics is same for all the three bacteria due to the addition with increasing concentration and incubation periods. Considering the soil, the angular shaped particles are typically more compressible because the sharp edges in the particles tend to be overstressed during increase in confining stress and shears as well during compression. In the treated samples, the increase in soil strength strengthened the bonding between the soil, which in turn increased bearing capacity of soil. Due to the presence of bacteria, which has the capacity in filling the internal pores in particles with a proper bonding with the calcite which improved the increasing confining stress, result in a reduction in deformation due to compressibility .

When compared with the untreated soil, the increased CBR values show that the *Bacillus* have high influence in imparting strength to the soil. It is mainly due to the availability of more influence of colony forms of *Bacillus* with clay which can react more during its reaction time of 4 days incubation anx soaking period.

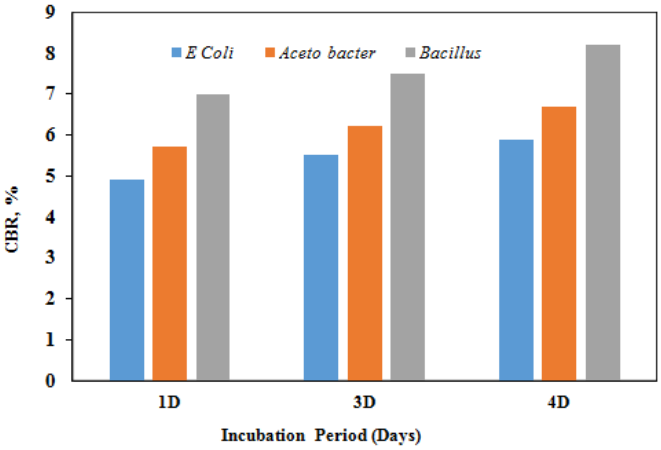

Figure 5.30 Variation of CBR value of soil treated with various bacteria for varying incubationperiods with concentration of 10^8 cfu/ml at 4 days soaking.

5.4. SWELL CHARACTERISTICS OF TREATED SOIL

The Free Swell determination is one of the effective method and generally adopted to identify the potential of swelling behavior in the soil and to estimate the expansive nature of soils. For conducting the free swell test, the samples were prepared for the UCC specimens. After the UCC tests, the samples were allowed to dry pulverized and utilized for free swell tests. The free swell index was conducted on both treated and untreated soils as per IS: 2720 (Part-40) specification.

For the Free swell index tests, soil were treated with *Acetobacter*, *Bacillus* and *E.coli* bacteria, a total of 135 samples are prepared under varying concentrations of 10^6, 10^7 and 10^8 cfu/ml, incubation periods of 1, 3, and 4 days with increasing curing periods of 1,7,14, 28 and 60 days. The free swell index results

with the bacteria *Acetobacter*, *Bacillus* and *E.coli* of varying concentration and incubation period are studied and are reported in Tables 4.8 to 4.10.

Table 5.8 Differential free swell value of soil treated with *Acetobacter*

Age of bacteria (Days)		1 Day (1D)			3 Days (3D)			4 Days (4D)		
cfu/ml										
Curing Days	1									
	7									
	14									
	28									
	60									

Table 5.9 Differential free swell value of soil treated with *Bacillus*

Age of bacteria (Days)		1 Day (1D)			3 Days (3D)			4 Days (4D)		
cfu/ml		10^6							10^7	
Curing Days	1									
	7									
	14									
	28									
	60									

Table 5.10 Differential free swell value of soil treated with *E. coli*

Age of bacteria (Days)		1 Day (1D)			3 Days (3D)			4 Days (4D)		
cfu/ml		10^6	10^7	10^8	10^6	10^7	10^8	10^6	10^7	10^8
Curing Days	1	80	75	75	70	65	60	60	60	55
	7	75	70	70	65	60	55	55	55	50
	14	70	65	65	60	55	50	50	45	45
	28	65	60	60	55	50	45	45	40	40
	60	60	60	55	50	40	40	45	40	35

From the Table 5.8 it was observed that FSI value of treated soil of *Acetobacter* concentration of 10^8 cfu/ml decreases to 30% compared with the untreated soil FSI value of 85% at the 4 days incubationperiod and curing age of 60 days. The increase in bacteria concentration decreases the FSI values for different incubation periods. Figure 5.31 and 5.32 shows the typical graphs representing the reduction in FSI value at changing curing periods for the treated samples with *Acetobacter* at 3 and 4 days incubation period.

Table 5.9 and 5.10 show the variation of FSI values of soil treated with *Bacillus* and *E.coli* and it can be concluded that with increase in bacterial concentrations, the free swell index decreases and that it decreases at nearly constant rate for the soil samples treated with both the bacteria. Similarly, for *Acetobacter* treated soil, the increase in curing period also decreases the free swell index values of soil.

The effect of age of bacteria and concentration of bacteria of *Bacillus* and *E.coli* on the treated soil is also compared with the free swell values. It can be seen that the FSI value of clay soil was decreased and its variation for the incubation periods of 3 and 4 days at increasing curing periods are presentedin Figures 5.33 to .36. This reduction in free swell index values of bacteria treated soil, provides an indirect indication of the strength improvement.

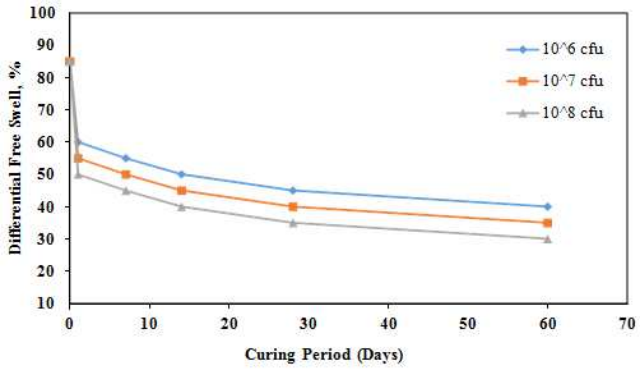

Figure 5.31 Effect of curing periods on FSI value for various concentration of *Acetobacter* at an incubation period of 3 days

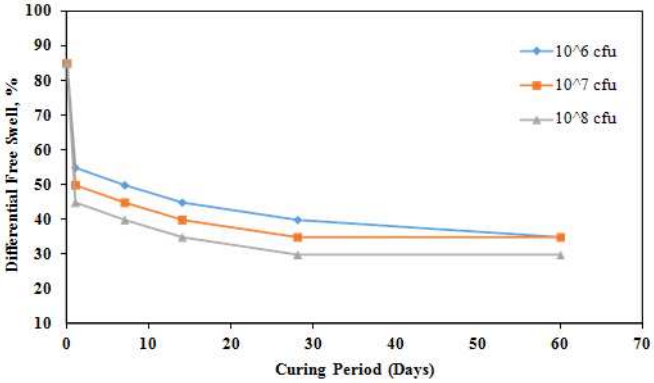

Figure 5.32 Effect of curing periods on FSI value for various concentration of *Acetobacter* at an incubation period of 4 days

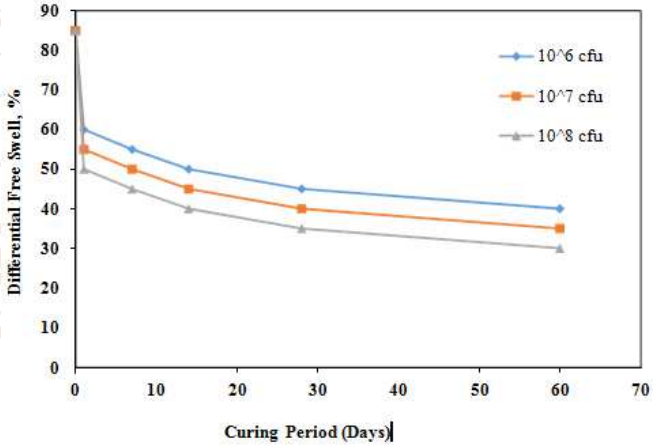

Figure 5.33 Effect of curing periods on FSI value for various concentration of *Bacillus* at an incubation period of 3 days

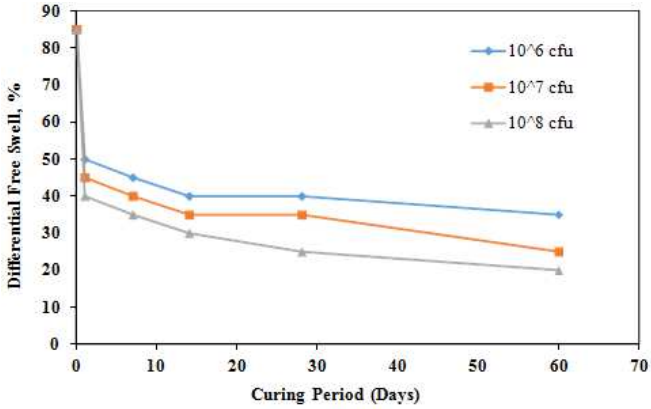

Figure 5.34 Effect of curing periods on FSI value for various concentration of *Bacillus* at an incubation period of 4 days

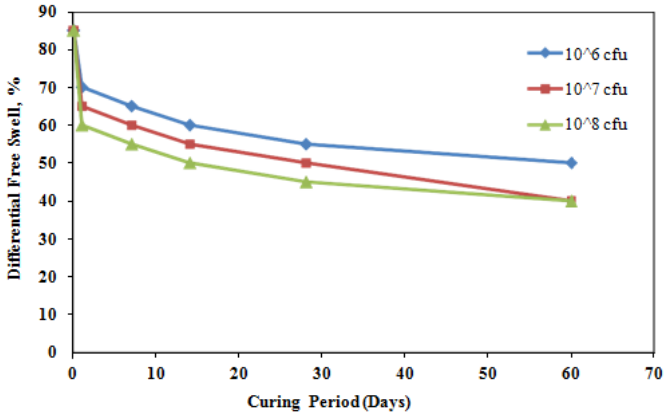

Figure 5.35 Effect of curing periods on FSI value for various concentration of *E. coli* at an incubation period of 3 days

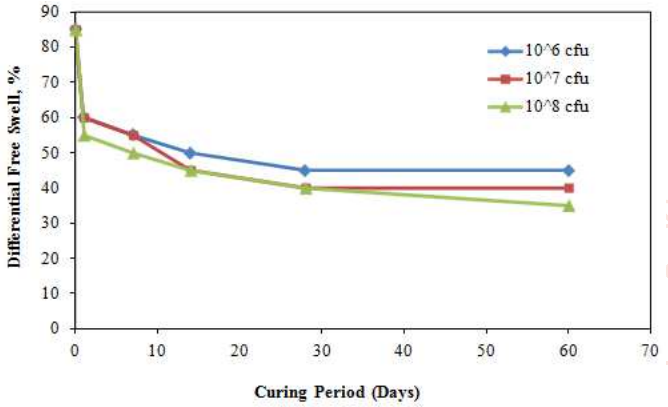

Figure 5.36 Effect of curing periods on FSI value for various concentration of *E. coli* at an incubation period of 4 days

From the results it is observed, when bacterial concentration increases, there is reduction in swell. As curing period increases results the reduction of the free swell index values. The percentage reductionis more for the treated soil with *Bacillus* than with *Acetobacter* and *E.coli* bacteria for the age of 4 days. The reduction in free swell values and the effect of type of bacteria on its reduction in the soil samples at 60 days curing period with 10^8 cfu/ml concentration were shown in Figure 5.37.

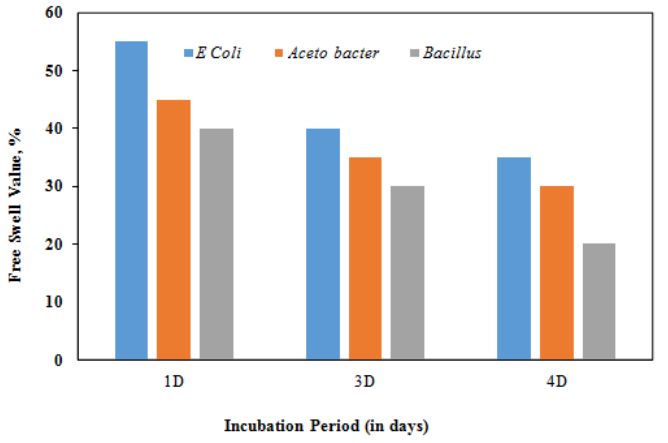

Figure 5.37 Effect of bacteria on FSI value of soil treated for various incubation period at 60 days curing period with 10^8 cfu/ml concentration

6. CONCLUSION AND FUTURE SCOPE OF WORK

6.1. GENERAL

In this study the expansive soil was treated with three types of bacterial cultures such as Acetobacter, Bacillus, and E.coli at different concentrations of the cells with different days of incubations and curing periods. Effectiveness of the usage of the MICP bacteria on strength behavior of soil were evaluated by conducting the strength tests, Un Confined compression (UCC) and CBR tests and swelling characteristics by Free swell index tests on treated soil. The micro structural analysis of calcite precipitating bacterial treated and untreated samples were analysed by SEM and element analysis by XRD.

6.2. CONCLUSIONS

Based on the virgin soil a property, the soil is classified as High compressible Clay (CH) and from the results of free swell and Atterberg's limits according to IS: 1498-1970, the degree of severity of thesoil is high to critical.

The calcite precipitating bacteria were isolated from the collected soils and they were identified as Acetobacter, Bacillus, E. coli based on the morphology and biochemical tests. The standard bacteria were purchased from microbial type collection center, Chandigarh, India for further studies.

The changes in UCC strength and FSI values were obtained for three concentrations of 10^6 cfu/ml, 10^7 cfu/ml and 10^8 cfu/ml, three incubation periods of 1, 3, and 4 days with soil and bacteria of Acetobacter, Bacillus and E. coli and for different curing periods of 1, 7, 14, 28 and 60 days.

The CBR tests on treated soil were conducted for three concentrations and incubation periods after 4 days soaking period.

From the UCS results conducted on soil treated with Bacillus incubated for 1, 3 and 4 days with concentrations 10^6,10^7, 10^8 cfu/ml, the percentage increase is about 280%, and swell got decreased from 85 to 20%. Considering the soil treated with *Acetobacter* and *E. coli* bacteria, the UCS values improved similar to *Bacillus*, but the improvement is in the order of 200% and 150% respectively. Similarly the free swell values are also decreases from 85 to 30 for soil treated with *Acetobacter* and 35 for the soil treated with *E. coli*.

From the CBR value of soil treated with *Acetobacter*, *Bacillus* and *E.coli* by considering all the incubation period and colony forming unit it was observed that the increase in CBR value is from

2.6 to 6.7, 8.2 and 5.9 respectively.

From the overall comparison and analysis of results it is observed that, UCS and CBR value increases as with increase in the age of bacteria (incubation period). This is because, the growth of bacterial cells increases for 24 h and further the growth is up to stationary phase of bacteria. Hence as the incubation period increases, the growth of bacteria increases and thereby increases the calcium precipitation which increases the CBR value and UCC strength. It is also observed that, the increase inconcentration of bacteria increases the UCS and CBR value.

Based on the effect of bacteria, the maximum percentage increase in the UCS and CBR and the swell reduction are observed in 4 days incubated *Bacillus* bacteria of 10^8 cfu/ml concentration when compared to *Acetobacter* and *E. coli* treated soil.

Microstructural studies like SEM and elemental analysis by XRD results also shows the improvement in morphology characteristics and calcium carbonate formation on treated

soil, which contributes strength development in stabilised samples.

It was observed that, there is an enhancement in the properties of the soil treated with MICP bacteria. Hence a MICP bacterium, which is cost-effective when compared to the cementitous stabiliser and it was non-intrusive, low energy demanding, pollution and waste free processes which can be used as an innovative sustainable materials for stabilising problematic soils.

6.3. SCOPE FOR FUTURE WORK

Further, it can be studied for the isolation of calcite precipitating bacteria and identification using 16s rRNA molecular based method. Also the calcite precipitating bacterial calcium may be quantified.

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